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Revisiting Aryl Amidine Synthesis using Metal Amide and/or Ammonia Gas: Novel Molecules and their Biological Evaluation

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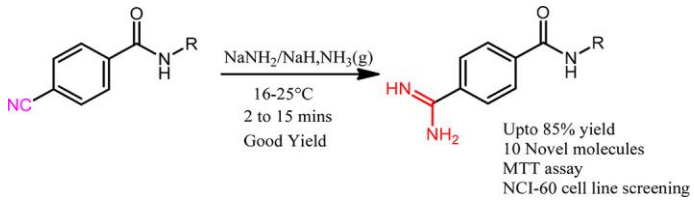
Abstract

Amidines, due to their unique biocompatibility and desirable physical characteristics, have been the functionality of choice as a scaffold for large number of drug synthesis. But still synthesis of amidines in presence of other active functional groups or pharmacophore, remained a challenge. In this work, a simple and reliable protocol for conversion of nitrile-amide to unsubstituted amidine-amide is developed using metal amide and/or ammonia gas. The scope and efficiency of this synthetic strategy is demonstrated on a number of substrates which differ in functional groups will be discussed. In this process ten novel aryl amidines in good yields (upto 85%) were synthesized. Biological evaluation revealed that compound 4-(aminoiminomethyl)-N-(2-furanyl methyl) benzamide (IC_{50}=9μM) and 4-(aminoiminomethyl)-*N*-(3-pyridinylmethyl) benzamide (73.36% growth inhibition) showed moderate efficacy for cancer cells.

Graphical Abstract



KEYWORDS: Amidine, amide, anti proliferative activity, single crystal, NCI

INTRODUCTION

Amidine functionality serves as synthon for the synthesis of variety of heterocyclic compounds. [1] On the other hand amidines with their unique structural properties and bio-compatibility are present in various active pharmaceuticals ingredients (API) employed for antiviral, anti-inflamatory and anticancer[2]. This latter aspect will open up new scope for amidines as a pharmacophore.

Syntheses of amidines have been explored by many scientists in the past. Out of the various reagents available for the synthesis of amidine, single step conversion of nitrile to amidine remained choice of synthetic chemists. In this method the nitrile functionality is activated by the electron withdrawing groups or lewis acids [3,4] to give the desired products.

Pinner reaction, to the best of our knowledge, happens to be the most commonly used technique for the amidine synthesis from the parent nitrile compound.[5] Contemplating

the mechanism, first step being the cyanide activation using dry HCl gas in dry methanol, leads to the formation of amidinates as shown in Scheme 1. Ammonia attack is the next step leads to amidine, the desired product, in good yield. Key step in this process remains the activation of nitrile group by HCl. This may lead to unwanted chemical reactions if additional functional groups are present on substrate. This is what is observed in our case, nitrile-amide system, where latter functional group started reacting prior to nitrile acitivation leading to the formation of undesired products. Hence for our substrate there is a need to find alternative methodology where nitrile group is activated prior to amide linkage.

Further literature search resulted in few more alternative methods where scientist have used different reagents/reaction conditions for the conversion of nitrile to amidine (Scheme 2).[3,6,7] Cornell et al (1928) reported the synthesis of aliphatic and aromatic amidines from their parent nitriles using liquefied ammonia gas with various metal amides.[8] Criticality of this reaction remained with the difficulty in handling liquefied ammonia gas in standard synthetic laboratory. Newbery et al (1947) used same reagent (metal amides) but with a slightly milder conditions, using benzene as solvent at high temperature.[9] The drawback of using this method was the solubility of the parent nitriles in benzene and also the toxicity of the solvent.[10] Interestingly, today's drug design challenge revolves around presence of more than one functional group (pharmacophores) on a single API.[11]

RESULTS AND DISCUSSIONS

Keeping above literature survey in mind, our efforts were focused on amidine synthesis in presence of amide ($R.CO.NH_2$) functionality, our substrates, using amide anion (NH_2^-). This process can be considered as a 'direct' amidine formation method, since it requires no prior activation of nitrile functionality. Now, NH_2^- can be generated in-situ as well as ex-situ means. Therefore we planned both these methods: Method 1- in-situ generation: dry NH_3(g) with sodium hydride in DMSO, and Method 2- ex-situ generation: Sodamide in DMSO (Scheme 3). The role of sodium hydride in the Method-1 is to abstract proton from ammonia and form NH_2^- (in-situ generation). Both these methods, when carried out at room temperature (16-25°C), gave good yields with substantial reduction in the time of the reaction as compared to literature. Interestingly bottle-neck for both these modified method remains in the contact time of sodamide and nitrile functionality, which can be controlled by tailoring the reaction conditions. Table 1 shows results of screening different reaction conditions for both these methods on 4-(aminoiminomethyl)-benzamide, a standard substrate with presence of amide functionality with nitrile. Thus, standardized optimum conditions were employed to synthesis of ten new compounds with amidine-amide linkages.

The synthesis of 4-(aminoiminomethyl)-benzamide, one of the standard lead compound, was carried out using Scheme 3. To validate this synthetic procedures different substituent's in the form of halogens, heterocycles were introduced near amide functionality. These structures are tabulated in table 1. While tailoring the structures with different substituent, PAINS (Pan Assay Interference Compounds) were kept in mind.[12] PAINS functionality doesn't discriminate between target and non-target

moieties leading to plethora of side effects. We observed key step during the synthesis of this series compounds remained in the formation of amidine from parent nitrile functionality.

4-iodo-*N*-(4-methoxyphenyl) benzamide **5a** was obtained from 4-iodobenzoic acid **4** (refer ESI for synthesis) in two stages, treatment with thionyl chloride in first step and DMAP/R-NH_2 [13–15] in second step (Scheme 2). For the later step, we employed variety of bases triethyl amine (TEA), diethyl amine (DEA) and (dimethyl amino pyridine) DMAP along with number of solvents such as dry ACN, MDC, $CHCl_3$ and CCl_4. The best yields were obtained with MDC and DMAP. Traditionally, Iodo/nitrile exchange employes NaCN or KCN but in our present strategy we preferred 'green' nitrile source in the form of cuprous cyanide (CuCN). Good yield of 4-cyano-*N*-(4-methoxyphenyl) benzamide **(6a-j)** were accessed by reacting 4-iodo-*N*-(4-methoxyphenyl) benzamide (**5a-j)** with the requisite CuCN as the nitrile source in dry DMF(refer ESI for detailed experimental procedure).

All the new compounds were characterized by FT-IR, ^{1}HNMR, ^{13}C NMR, Mass spectrometry and micro analysis (Refer ESI for spectral details). Spectra analyses were consistent with the assigned structures.

Figure 1 shows ORTEP diagram for single crystal of 7j. It crystallizes in triclinic crystal system with P1 space group, with two molecules in an asymmetric unit. No prominent hydrogen bonding is observed in spite of having presence of strong hydrogen bond donor

and hydrogen bond acceptor groups. Significant and expected short contact amongst two neighboring amide bonds through NH···O bonding (3.247Å and 171.5°) is observed along *a*-axis. Although this interaction is considered to be weak in nature but it is reported that it plays an important role in the protein-drug binding.[16] This short-contact also helps in maintaining planarity of two aromatic groups and hence pharmacophore. (CCDC no. 1432792, detailed structure information can be obtained from supporting information).

Cytotoxicity studies[17] on HeLa cell line was performed for all the new compounds. Cis-platin was used as the reference drug. Percentage cell viability of synthesized compounds on HeLa cell line at various concentrations was checked and then from that IC_{50} was calculated (One way ANOVA (non-parametric test was carried out. P value= 0.0062(**)). Table 3 shows results of IC_{50} in µM concentrations.

All molecules were initially screened for anti-proliferative activity *in silico* by National Cancer Institute (NCI), USA (Refer ESI for experimental procedure). Out of this, compound 7b, 7c, 7d and 7g were further selected for actual screening anti-proliferative activity at 10µM concentration. Graph 1 shows comparative study of compound 7b, 7c, 7d and 7g on selected human derived cell lines NCI-H522 (Non-Small cell lung cancer), HCT-116 (Colon cancer), SF-539 (CNS cancer), OVCAR-8 (Ovarian cancer) and SN-12 (Renal cancer). 7g shows 73.36% growth inhibition in HCT-116 colon cancer cell line (mean growth inhibition) at 10µM concentration.

Two heterocyclic structure containing derivatives of furan and picolyamine were found to be most potent among all. Both compounds 7g and 7h have a heterocycle in conjugation with NH side of amide linkage, but also have flexible -CH_2 bridge. Thus, from anti cancer activity perspective we can conclude that 4-(aminoiminomethyl)-*N*-(3-pyridinylmethyl)benzamide **(7g)** and 4-(aminoiminomethyl)-*N*-(2-furanylmethyl)benzamide **(7h)** can be investigated further for the development as new leads.

EXPERIMENTAL

This includes synthesis and characterization of one of the new molecules. Experimental procedures for the intermediates and rest of the derivatives are presented in supporting information.

Materials And Methods

All the compounds were purified using column chromatography (2000- 400 mesh silica) before characterization. TLC analysis was done using pre-coated silica on aluminum sheets. Melting points were recorded in Thiele's tube using paraffin oil and are uncorrected. FT-IR (KBr pellets) spectra were recorded in the 4000-400 cm^{-1} range using a Perkin-Elmer FT-IR spectrometer. The NMR spectra were obtained on a Bruker AV-III 400 MHz spectrometer using TMS as an internal standard. The chemical shifts were reported in parts per million (ppm), coupling constants (J) were expressed in hertz (Hz) and signals were described as singlet (s), doublet(d), triplet(t), broad (b) as well as multiplet (m). The microanalysis was carried out using a Perkin-Elmer IA 2400 series

elemental analyzer. The mass spectra were recorded on Thermo scientific DSQ-II. All chemicals and solvents were of commercial grade and were used without further purification. Single crystal data was collected with Xcalibur, EoS, Gemini.

General Procedure For The Synthesis Of Compound 7

Method 1: Dry DMSO and NaH(60% suspension in oil, 0.100mg,0.0025 mol) were stirred at 16-20°C for 15mins. Compound 6a (0.100g, 0.0037mol) was then added and ammonia gas was purged in it till the completion of the reaction (monitored by TLC, eluent, petroleum ether/ethyl acetate, 1/4, V/V), cooled the reaction. Then the water was added slowly such that temperature of reaction should not exceed 30°C. Residue was extracted with ethyl actetate (3x10ml) and purified by neutral alumina column chromatography (eluent, petroleum ether/ ethyl acetate, 1/4, v/v) to afford compound 7 as off white solid to pale yellow solid. Yield: 60-85%. Melting point: 200-202°C.

Method 2: Dry DMSO and compound 6a (0.100g, 0.0037mol) were stirred at 25°C for 5mins, added sodamide in it and stirred for 2-15mins at same temperature. Reaction was monitored by TLC (eluent, petroleum ether/ethyl acetate, 1/4, v/v), cooled the reaction and water was added slowly such that temperature of reaction should not exceed 30°C. Residue was extracted with ethylacetate (3x10ml) and purified by neutral alumina column chromatography (eluent: petroleum ether/ethylacetate, 1/4, v/v) to afford compound 7 as off-white solid to pale yellow solid. Yield: 60-85%.

4-(Aminoiminomethyl)-*N*-(4-Methoxyphenyl) Benzamide (7a)

Following the above general procedure title compound was synthesized. Off-white solid. Yield: 82%; M.P: 200-202°C ;IR (KBr) γ: 3336.77(amidine-NH), 2923 (w), 1681.31(C=O), 1647.57(C=N), 1534.10(NH), 1269.39(C-N), 1249.80(C-O), 1031.31(C-O amide), 824.13(*para* substitution) cm-1; ^{1}H NMR (400MHz,DMSO-d6) δ: 10.37(s, NH), 8.04(d,2H, J=8 Hz), 7.99(d,2H,J=8 Hz),7.59(d,2H,J=8.8 Hz), 6.92(d,2H,J=8.8 Hz), 3.75(s,3H), 2.65(s,3H, amidine) ppm; ^{13}C NMR (100MHz, DMSO-d6) δ: 198.9(C=NH), 165.3(C=O), 156.4(C-O), 139.1(C-C=O), 131.8(C-C=NH), 128.7(2C), 128.3(2C), 122.9(2C), 114.3(2C), 55.6(O-CH_3) ppm; MS(m/z): (M^+) 269.15; Micro Analysis: Anal. Calc. for $C_{15}H_{15}N_3O_2$: C, 66.90; H, 5.61; N, 15.60 %; Found: C 67.20; H, 5.40; N, 15.82 %.

CONCLUSION

Conversion from nitrile to amidine can be achieved effectively in a single step and in the presence of amide functionality using metal amide and/or ammonia gas. This method is extended for the synthesis of ten new amidines-amide conjugates where strategy works effectively in presence of heterocyclic functionality as well. Apart from nearing room temperature most of the time yield observed in the modified reaction conditions clocks above 70% for nitrile to amidine conversion. Our preliminary results confirmed that present strategy, amidine-amide conjugates can act as anti-proliferative active compounds, similar to observed in literature.[18] In short, this study paves a way to synthesize not only novel amidines but also amidine-amide conjugates, a strategy for future drug design.[19]

SUPPLEMENTARY DATA

Supplementary data (experimental procedures and full spectroscopic data for all new compounds) associated with this article can be found, in the online version. CCDC 1432792 (7j) contains the supplementary crystallographic data for this paper. These data can be obtained from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data request/cif.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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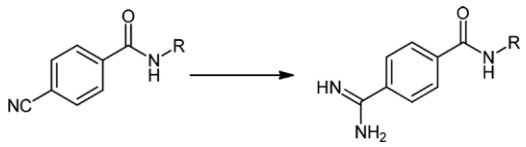
Table 1 Investigation of solvents and reagents on our substrates



Entry	Reagent[b]	Solvent	Yield[c] %
1	Na, NH_4Cl	MeOH	NR
2	HCl(g), EtOH/NH_3(g)	EtOH	NR
2	NH_2OH.HCl,TEA/NH_3(g)	EtOH	NR
3	NH_4Cl, Si[d]	MeOH	NR
4	EtOH.HCl/NH_4Cl	MeOH	NR
5	NH_3(g), NaH	THF	15
6	NH_3(g), NaH	DMF	35
7	NH_3(g), NaH	Toluene	NR
8	NH_4Cl, NaH	Toluene	NR
9	NH_4Cl, NaH	DMSO	NR
10	NH_3(g), NaH	DMSO	83
11	$NaNH_2$	DMSO	85

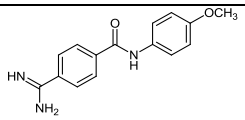
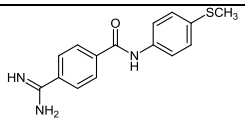
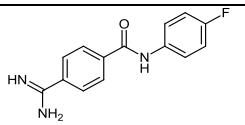
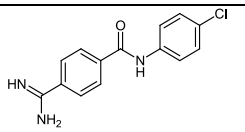
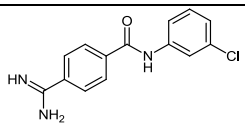
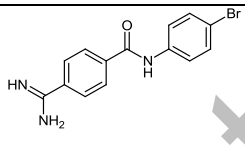
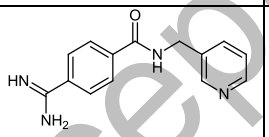
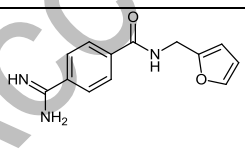
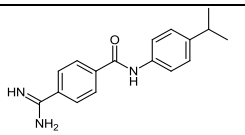
*NR= No reaction, desired product is not obtained.

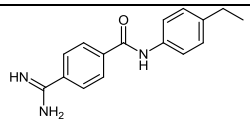
[b] Reactions were performed based on the literature procedure.

[c] Yield of the isolated product after silica gel chromatography

[d] Reaction was performed in a sealed tube.

Table 2 List of derivatives of amidine-amide conjugates (7)

Entry	Structure (R)	Yield*	Melting Point(°C)
7a		82	200-202
7b		85	230-232
7c		74	204-205
7d		78	198-200
7e		70	135-140
7f		60	220-222
7g		65	156-158
7h		75	144-146
7i		83	170-172

7j		72	180-183

*Yields are reported for the final step f

Table 3 IC_{50} Values of the compounds 7a-j on HeLa cells

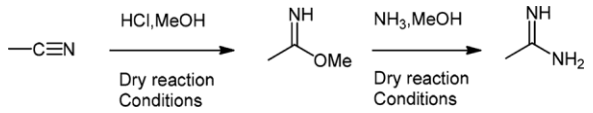
Compound	7a	7b	7c	7d	7e	7f	7g	7h	7i	7j	Cisplatin
$IC_{50}(\mu M)$[a]	>200	189	>200	153	>200	ND[b]	>200	9	>200	ND[c]	28

[a] IC_{50} values are the mean of four independent determinations,

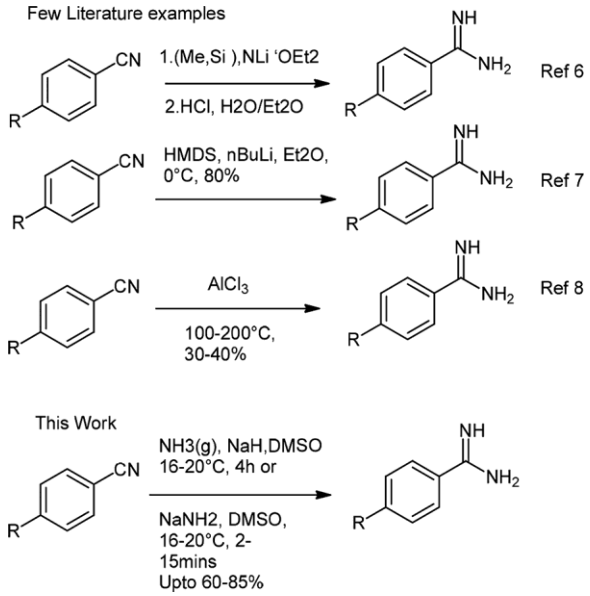
[b] IC_{50} value exceeded the mM concentration,

[c] Poor solubility

Scheme 1. Pinner Reaction condition



Scheme 2. Literature work for amidine synthesis



Scheme 3. Synthesis of target 4-(aminoiminomethyl)-benzamide derivatives

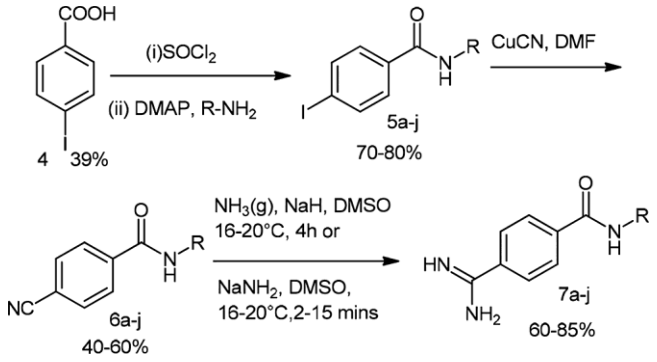
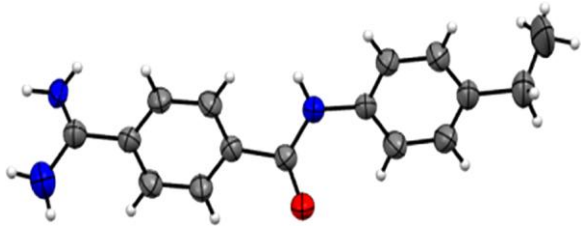


Figure 1. ORTEP diagram of 7j with 50 % probability



Graph 1. % Growth Inhibition on selected cell lines at 10µM concentration NCI-H522: Non-Small cell lung cancer cell line; HCT-116: Colon cancer cell line; SF-539: CNS cancer cell line; OVCAR-8: Ovarian cancer cell line; SN-12: Renal cancer cell line